Endotoxin Fever and Tolerance in Totally Adrenalectomized Rabbits[1] (35301)

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Adrenalectomy markedly enhances susceptibility to the lethal activity of bacterial endotoxin (1–6). Whereas protection of adrenalectomized animals is afforded by adrenal cortical extracts, deoxycorticosterone acetate (DOCA) confers minimal (4) or no protection (5). In 1950, Grant and Hirsch (7) studied the effects of adrenalectomy on endotoxin-induced fever. These investigators concluded that in contrast to lethality, the course of pyrogen-induced fever in rabbits is very little affected by adrenalectomy when the animals are maintained healthy by DOCA (7). In these studies, however, it was emphasized that no attempt was made to remove all adrenal tissue. In addition, adrenalectomized animals were injected intravenously with the same endotoxin preparation prior to surgery to establish base line fever levels; some animals were injected twice. The possibility of protection from residual tolerance was not excluded. Finally, no indication was given that testing was carried out with doses of endotoxin that evoked fever within the sensitive portion of the dose–response range. The present studies were performed to quantitate the effects of total adrenalectomy in rabbits never previously injected with endotoxin employing toxin doses that evoke fever within the sensitive portion of the dose–response range. The effects of total adrenalectomy on the development of endotoxin tolerance and on anti-"O" antibody formation are also quantitated.

Materials and Methods. Female albino rabbits weighing approximately 2 kg, raised on antibiotic-free feed, were obtained from a uniform source of supply. All syringes, needles, and glassware were either disposable, sterile, and pyrogen-free (Burron Medical Products, Inc., Bethlehem, Pa.), or heated overnight in a dry-air oven at 200° to eliminate extraneous pyrogens. Two hr before anesthesia, 5 mg of cortisone acetate (Intra Products, Inc., Dayton, Ohio) was given im. The animals were anesthetized with pyrogen-free sterile pentobarbital (Diabutal, Diamond Laboratories, Inc., Des Moines, Iowa). The shaven abdominal wall was treated with 2% tincture of iodine, washed with 70% alcohol, and covered with sterile drapes. All instruments were preheated overnight at 200° to eliminate extraneous pyrogens. Bilateral total adrenalectomy was performed by the technique described by White (8). Sterile DOCA pellet, 75 mg, (Cortate, Schering Corporation, Bloomfield, N.J.) was implanted subcutaneously. Incisions were closed with sterile silk sutures. 30 min postoperatively, 5 mg of cortisone acetate was given im, and then 3 mg was given daily for 3 days. Skin sutures were removed on seventh postoperative day. Three to 4 weeks postoperatively, the animals were acclimatized for fever studies. Sham-operated animals were treated identically except that the adrenals were left intact. Postmortem examination upon termination of the studies confirmed the completeness of adrenalectomy.

All pyrogen studies were conducted in animals loosely restrained by chain collars in fiber glass stalls after acclimatization for 18 to 24 hr. Temperatures were monitored with thermistor probes inserted 6 in. into the rectum and connected to a recording telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). Temperatures were de-

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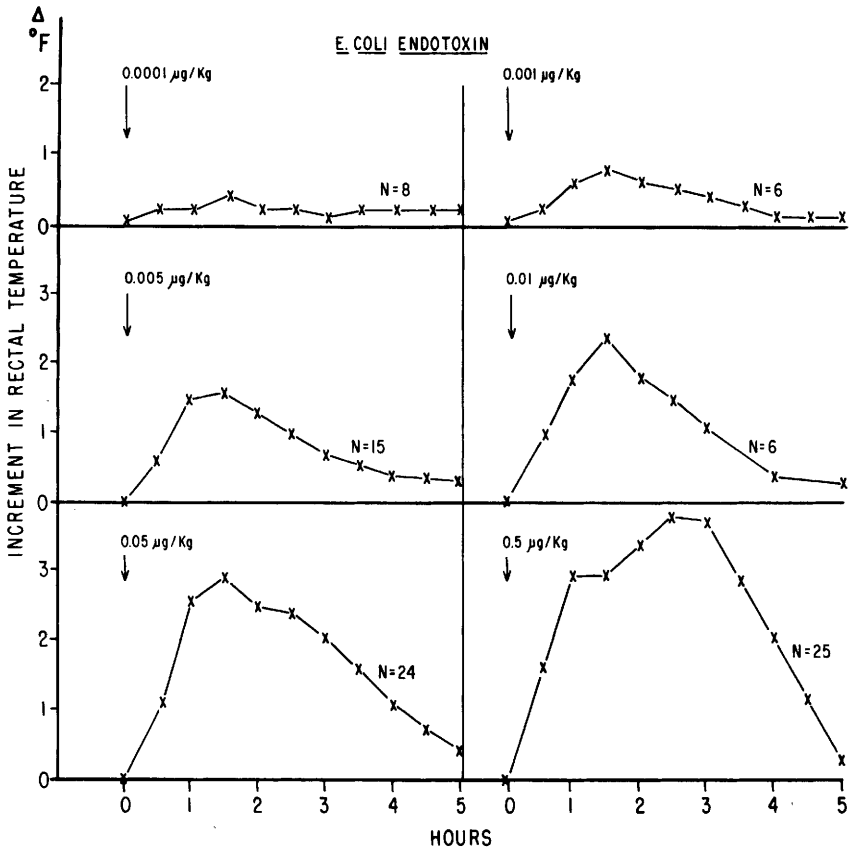

FIG. 1. Relationship between mean febrile response and iv dose of *E. coli* endotoxin in healthy New Zealand rabbits.

termined for 1 to 2 hr before each experiment; animals exhibiting initial levels above 104°F or varying more than 0.3°F in any 0.5-hr period were excluded.

Escherichia coli endotoxin, 0127B8, a Boivin type preparation (Difco Laboratories, Detroit, Mich.) was injected into a marginal ear vein. Temperatures were followed every 30 min for 5 hr.

Steroid assay. To demonstrate the thoroughness of adrenalectomy, stimulation with adrenocorticotropin (ACTH) was performed. Two-ml base line heparinized plasma samples were obtained at the completion of the pyrogen studies. ACTH (Highly Purified Acthar gel, Armour Pharmaceutical Co., Chicago, Ill.) 4 units, was then administered im to each rabbit, every 12 hr, for a total of 3 days. A second plasma sample was obtained; and corticosterone levels were determined on paired plasma specimens by the method outlined by Beitins *et al.* (9).

Anti-O antibody titers. These were assayed by the bentonite flocculation technique described by Wolff *et al.* (10).

Results. Three to 4 weeks postoperatively, animals gaining weight and appearing healthy were selected for study. Five adrenalectomized and 5 sham-operated control rabbits meeting these criteria were given increasing daily iv doses of *E. coli* endotoxin for 1 week. Endotoxin doses were carefully chosen so as to always induce fever within the sensitive portion of the dose–response range, Fig. 1. Figure 2 summarizes the results. Adrenalectomized rabbits did not hyperreact to the initial (Day 0) endotoxin iv injection; indeed, their initial response was slightly, although not significantly, blunted. Subsequent febrile responses also were not significantly

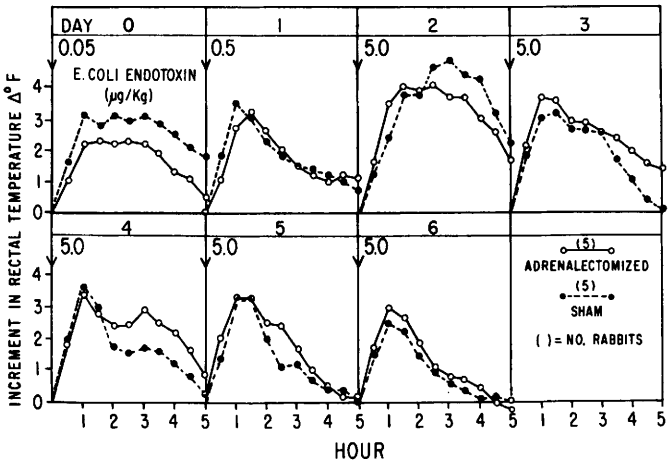

FIG. 2. Comparative mean febrile response of 5 totally adrenalectomized and 5 sham-operated rabbits to daily *E. coli* endotoxin iv injections.

different from sham-operated controls, *i.e.*, tolerance developed similarly to nonadrenalectomized animals.

Corticosterone plasma levels in adrenalectomized and sham-operated rabbits were measured before and after ACTH stimulation. The presence of some low levels of corticosterone in the adrenalectomized animals can be attributed to (a) the exogenous DOCA, and (b) methodology ("blank" effect). The key finding was that in contrast to sham-operated animals, adrenalectomized animals failed to respond to ACTH, verifying on a physiologic level the effectiveness of adrenal extirpation, Table I.

Anti-O antibody titers were measured on day 7 in adrenalectomized and sham-operated rabbits that received *E. coli* endotoxin according to the dose schedule outlined in Fig. 2. No appreciable differences in the geometric mean titers were observed, Table II.

TABLE I. Corticosterone Plasma Levels (μg/100 ml).

Rabbit	Base line	After ACTH
	Adrenalectomized	
A_1	0.9	1.2
A_2	0.9	0.9
A_3	0.3	0.4
A_4	1.2	1.0
Mean	0.82	0.87
	Sham-operated	
S_1	2.5	3.8
S_2	1.6	4.4
S_3	0.7	10.4
S_4	0.4	3.2
Mean	1.3	5.45

TABLE II. Anti-O Antibody Titers to *E. coli* Endotoxin.

Rabbit	Antibody titer
Adrenalectomized	
A_1	1:4
A_2	1:64
A_3	1:128
A_4	1:128
A_5	1:64
Geometric mean	1:48
Sham-operated	
S_1	1:256
S_2	1:32
S_3	1:256
S_4	1:2
S_5	1:32
Geometric mean	1:42

Discussion. Adrenalectomy markedly enhances susceptibility to the lethal activity of bacterial endotoxin (1–6). Enhancement of susceptibility persists even when the animals are maintained on DOCA (4, 5). The present studies demonstrate that completely adrenalectomized rabbits maintained on DOCA exhibit no enhanced susceptibility to the pyro-

genic activity of bacterial endotoxin. The dosage of endotoxin was carefully selected so as to evoke febrile responses within the sensitive portion of the dose–response range. Determinations of plasma corticosterone levels before and after ACTH stimulation, verified on a physiological level, the effectiveness of the adrenalectomy. It can be firmly concluded, therefore, that when mineral balance is maintained with DOCA, endogenous adrenal cortical or medullary secretions play no important role in the febrile response to bacterial endotoxin. These findings thus confirm those of Grant and Hirsch (7). Similarly, the present studies confirm the later preliminary report of Grant and Hirsch (11) that adrenalectomized rabbits maintained on DOCA develop pyrogenic tolerance to bacterial endotoxin in a normal fashion. In the present studies, unmodified tolerance acquisition was confirmed employing increments in endotoxin dosage carefully selected to induce febrile responses within the sensitive dose–response range.

Studies on the influence of adrenalectomy on antibody synthesis are conflicting. Depression (12), enhancement (6, 13–15), and no effect (3, 16–18) have been reported. The present studies demonstrate no significant effect of complete adrenalectomy on production of anti-O antibodies to *E. coli* endotoxin in rabbits maintained healthy with DOCA.

Summary. The initial febrile response, the acquisition of pyrogenic tolerance, and the titer of anti-"O" antibody to a Boivin preparation of *E. coli* endotoxin was not significantly altered in totally adrenalectomized rabbits maintained healthy with DOCA.

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